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Spatial and temporal patterns of distribution of the gap junction protein connexin43 during mouse gastrulation and organogenesis

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Summary

Connexin43 (Cx43) is a member of the family of channel-forming proteins that make up the gap junction and are believed to provide pathways for cell-cell exchange of developmental signals. We have used immunofluorescence and confocal microscopy to characterize the patterns of distribution of Cx43 in postimplantation mouse embryos representing stages of development extending through gastrulation and the major period of organogenesis [through 13.5 days post coitum (dpc)]. We find that Cx43 is expressed early after implantation by the undifferentiated, pluripotent cells of the primitive embryonic ectoderm from which all tissues of the fetus are believed to be derived. As cells become committed to particular developmental pathways, there is a progressive restriction of Cx43 to specific areas and organ systems. The patterns are complex and not limited by germ layer of origin, although there is a clear preference for expression in ectodermal and, to a lesser extent, mesodermal derivatives. Expression in lens, retina, kidney, brain, pineal and pituitary glands is initiated early in organogenesis. In heart, the first clear signal for Cx43 appears in the ventricle at about 10 dpc and is only subsequently detected in the atrium at about 13-13.5 dpc. Particularly intriguing with regard to functional implications is the high level expression observed at sites of inductive interaction; the eye lens and optic cup, the infundibulum and the apical ectodermal ridge of the limb bud.

Key words: connexin43, gastrulation, gap junction, inductive interaction sites.

Introduction

Cells of multicellular organisms and their embryos are interconnected by gap junctions composed of members of a family of proteins known as connexins (Cx) (Beyer et al., 1987; Beyer, 1990). Gap junctions provide pathways of communication believed to be crucial for normal embryonic development [reviewed by Revel (1986), Green (1988), Warner (1988), Guthrie and Gilula (1989)]. The most convincing evidence that gap junctional channels are utilized for morphogenetic signalling is often considered to come from experiments in which developmental abnormalities have followed the use of anti-Cx antibodies or antisense constructs (Warner et al., 1984; Fraser et al., 1987; Lee et al., 1987; Bevilacqua et al., 1989; Allen et al., 1990). Unfortunately, it has seldom been unequivocally demonstrated that the probes used in such experiments were specific for the connexin(s) expressed at the particular time or place. Ambiguities in the interpretation of these experiments have been exacerbated by an increasing awareness of the complexity of the connexin family (Paul, 1986; Kumar and Gilula, 1986; Beyer et al., 1987; Kistler et al., 1988; Zang et al., 1989; Beyer, 1990; Gimlich et al., 1990; Hoh et al., 1991) and by the fact that more than one connexin gene may be expressed in a given tissue or even in a single gap junction (Nicholson et al., 1987). It is therefore important to obtain precise information on the patterns of expression of the different connexin genes. With this goal in mind, we have used immunocytochemistry to determine the spatial and temporal patterns of distribution during gastrulation and organogenesis in the mouse embryo of a widely distributed member of the connexin family, Cx43 (Manjunath et al., 1987).

Materials and methods

Preparation of embryos

Embryos of specific ages were obtained by using mice (BALB/c, Simonson Laboratories, Gilroy, CA) where the pregnancy had been timed by the supplier, or, in the case of younger embryos, where the mating followed superovulation induced by injection of pregnant mare's serum (Sigma, St. Louis, MO) followed by human chorionic gonadotropin (Sigma, St. Louis, MO), according to Hogan et al. (1986). Embryos were processed for paraffin embedding as described previously (Yancey et al., 1988). When not obscured by the

decidua, the embryos were oriented for saggital, frontal, and transverse sectioning. The age of the embryos was determined according to the convention (Hogan et al., 1986) that fertilization takes place at midnight. Noon on the next day is 0.5 days post coitum (dpc). Staging was confirmed according to Rugh (1968). Embryos were examined at approximately 0.5 to 1-day intervals from 5.5 to 13.5 dpc.

Immunofluorescence

The polyclonal antiserum was raised in a rabbit against a synthetic peptide representing amino acids 360-382 located at the carboxy terminus of the Cx43. It is highly specific for Cx43 (Laird and Revel, 1990: Yancey, unpublished observations). Serial sections of the embryos were processed for immunocytochemistry as described (Yancey et al., 1988). Primary and secondary antibodies were each diluted 1:500. Sections were examined by epifluorescence on a Zeiss microscope and also on a Zeiss confocal microscope.

Results

In the following descriptions, we have equated a punctate pattern of fluorescence at the cell surface with the presence of gap junctions. Our rationale for this assumption is based on the specificity of our antibody and the fact that immunocytochemistry has revealed little evidence for a punctate appearance in intracellular pools of Cx43 in heart cells in culture or in a large number of adult tissues under normal conditions (Laird and Revel, 1990; Yancey, unpublished). The assumptions underlying our work are presented in some detail in the discussion section.

Early egg cylinder stage through gastrulation, 5.5-8.5 dpc

The earliest stage of embryogenesis examined corresponds to about 5.5 dpc, implantation occurring 4.5 to 5 dpc. At this time the embryo is a cylindrical structure (the egg cylinder) consisting of two layers, the primitive endoderm and ectoderm derived from cells of the inner cell mass. Cx43 is detectable, but not abundant, in both layers but predominantly in the primitive endoderm (Fig. 1A), which will not contribute to formation of the fetus. At this and subsequent stages, sections treated with preimmune serum or with secondary antibody in the absence of the primary antibody exhibited no punctate labeling (data not shown).

With further differentiation of the egg cylinder, the ectoderm comes to line the proamniotic cavity, and the cylinder becomes divided into embryonic and extraembryonic areas (Fig. 1B). By this time, the number of labeled cells has markedly increased and there is a differential distribution of Cx43. In the extraembryonic portion the signal is most intense in the endoderm, but in the region that will become the embryo the signal is nearly all confined to the ectoderm, from which the tissues of the fetus originate. Only changes occurring in the embryo proper will be further detailed in this report.

Gastrulation begins at about 6.5 to 7 dpc as cells begin to ingress from the posterior part of the embryonic ectoderm to form the mesoderm. The cells

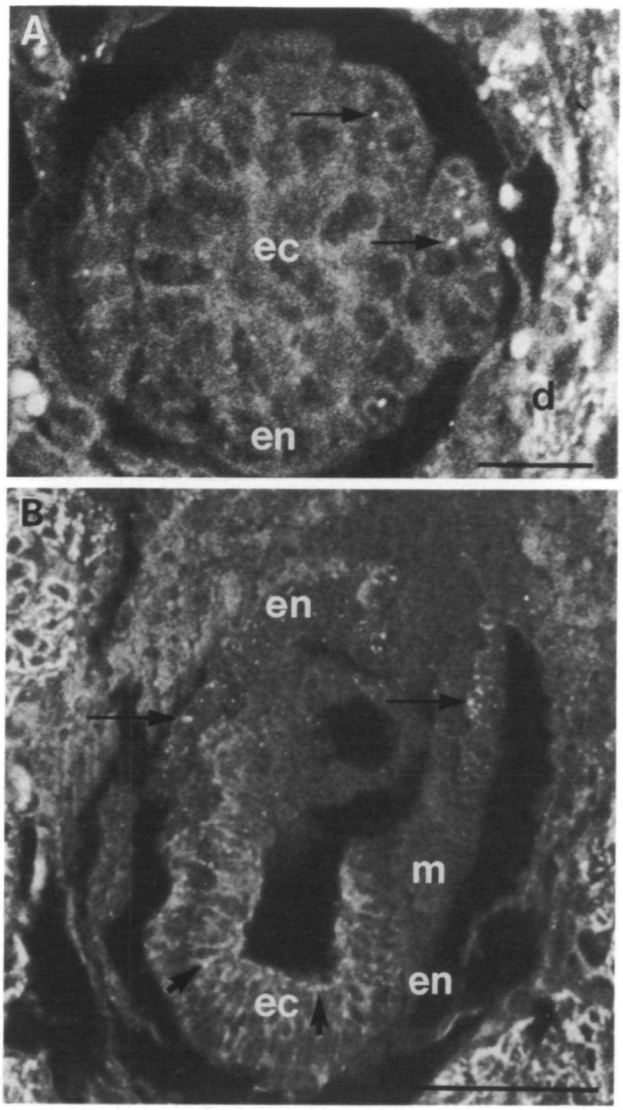


Fig. 1. Distribution of Cx43 immunoreactive protein in confocal microscope views of early postimplantation embryos. (A) Transverse section of embryo, approximately 5.5 dpc. At this stage the embryo consists of only two cell layers, the primitive ectoderm (ec) surrounded by the primitive endoderm (en). A few fluorescent puncta (arrows) indicate low level expression of Cx43 in both layers but predominantly in the primitive endoderm. In contrast, the cells of the surrounding decidua (d) are heavily labeled. Bar, 25 μm. (B) Longitudinal section of embryo, approximately 7 dpc. The site of implantation is at the top. The embryo can now be subdivided into extraembryonic (top half) and embryonic tissues (bottom half) which surround the proamniotic cavity. A layer of mesoderm (m) has begun to form through ingression of cells from the ectoderm. In the extraembryonic region, Cx43 containing junctions (long arrows) are prevalent in the endoderm (en); however, in the embryonic half, the junctional connections (short arrows), although present in all three layers, are most abundant between the cells of the ectoderm (ec), the cell layer from which all the diverse tissues of the fetus are believed to originate. Bar, 100 μm.

of the mesoderm are labeled by the antibody but at a low level relative to the cells in the ectoderm. The primitive endoderm is only weakly labeled and has become flattened and tenuous, presaging its eventual disappearance. The definitive endoderm is believed to be formed from the ectoderm during later stages in gastrulation (reviewed by Beddington, 1983).

By about 7.5 to 8 dpc (Fig. 2A,B), fluorescent puncta denoting the presence of Cx43-type gap junctions are seen in all three germ layers. They are increased both in size and number throughout the ectoderm. Part of the latter has thickened to form the neural plate which rolls up to form the neural tube. The labeled gap junctions, however, appear to be homogeneously distributed within the ectoderm (Fig. 2A,B). In contrast, the staining of the mesoderm varies depending upon where in the embryo one looks, appearing primarily in the mesenchyme located in posterior regions and in the somites (Fig. 2B). Some cells located in the region of the heart primordium, the primitive heart tube, are also stained (Fig. 2A). The cells that line the foregut indentation, presumably definitive endoderm (Rugh, 1968; Beddington, 1983), are intensely labeled (Fig. 2A).

Organogenesis, 9-13.5 dpc

During organogenesis, the distribution of Cx43 becomes restricted to specific organ primordia and specific regions of the developing brain and spinal cord. It is, however, widely distributed. For the sake of simplicity, the results from this series of embryos are summarized in Table 1, with only the salient features described in detail below.

Differential patterns of distribution of Cx43 in developing brain regions

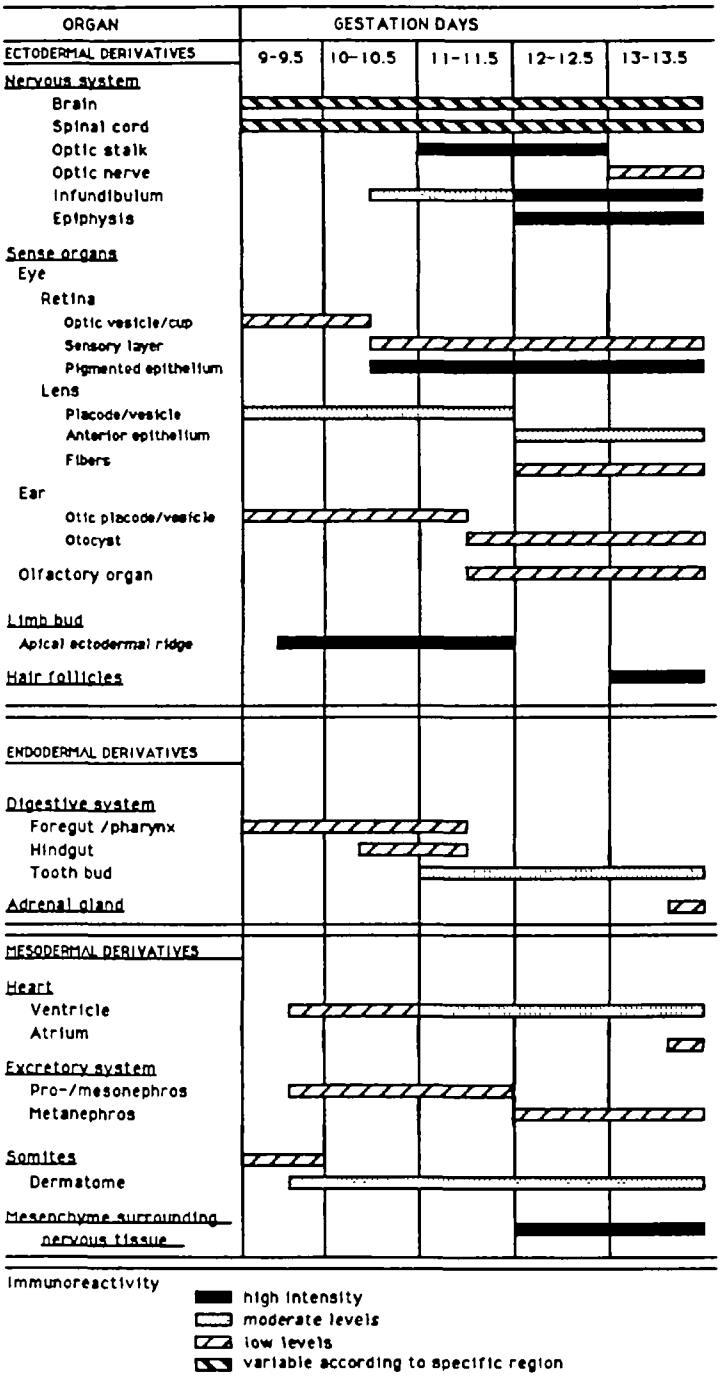
The differentiation of the brain vesicles (9-10 dpc) is accompanied by a dramatic shift in the localization of Cx43 away from the apparently homogeneous distribution seen in the neural plate and tube at 8-8.5 dpc. By 9.5 dpc, Cx43-type gap junctions are distributed throughout the diencephalon, and in a circumscribed area of the telencephalon, but are absent from most other areas (Fig. 3A).

A number of structures are produced by evaginations of the diencephalon, including the optic vesicles, the infundibulum and the epiphysis. The Cx43 gene is expressed in all of these structures even when expression in the diencephalon itself, maximum at about 9-5 dpc, has been markedly reduced.

The infundibulum, part of which becomes the neural lobe of the pituitary gland, is formed as an outpouching of the floor of the diencephalon beginning at about 10.5-11.5 dpc (Rugh, 1968) as it comes in contact with Rathke's pouch. As the infundibular evagination enlarges and elongates, it becomes increasingly labeled by the anti-Cx43 antibody so that by 12.5-13.5 dpc it is one of the most intensely labeled structures in the embryo (Fig. 3B). On the other hand, Rathke's pouch is not labeled.

The epiphysis, the precursor of the pineal gland, begins to pinch off from the diencephalon by about 13.5 dpc (Fig. 3C). It too is heavily labeled by the anti-Cx43 antibody, with junctions concentrated near the apex of the cells.

Table 1. *Expression of connexin43 during organogenesis*



Development of the eye and optic nerve

By about 9.5 dpc, the optic vesicles, which arise as outgrowths from the lateral wall of the diencephalon, have made contact with the surface ectoderm. In response, the ectodermal cells overlying the optic vesicle elongate, forming the lens placode (Fig. 4A).

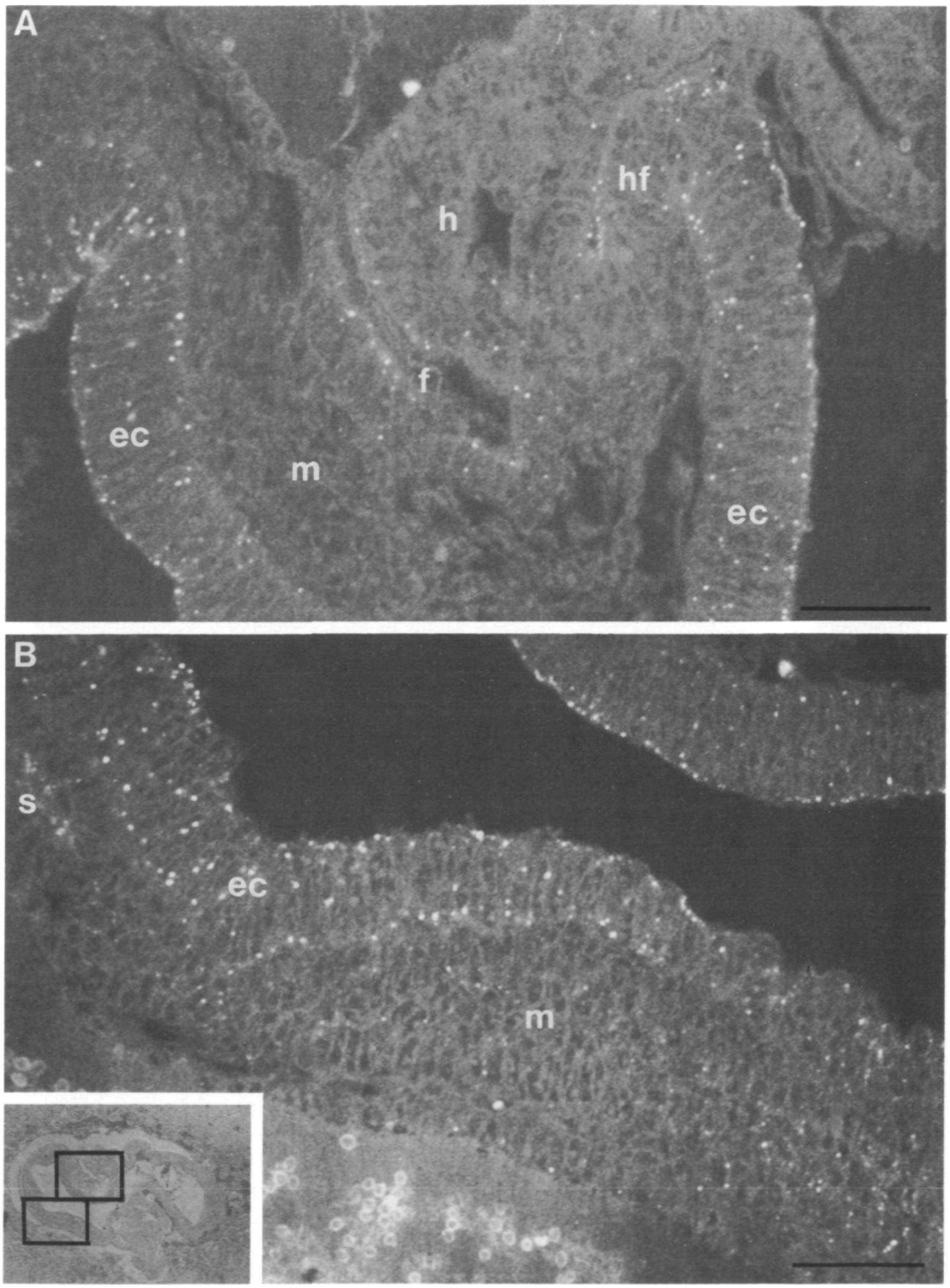


Fig. 2. Longitudinal section of the embryo, 7.5-8 dpc. The insert shows a view of the entire embryo, embryonic half oriented to the left, extraembryonic half and site of implantation to the right. The boxed areas are shown at higher magnification in confocal images in A and B. (A) Anterior region. Cx43 type junctions are distributed throughout the ectoderm (ec), including that of the headfold (hf). They also interconnect the endodermal cells lining the foregut (f). Mesoderm (m), except for that in the region of the heart primordium (h), is negative. (B) Posterior region. As in all regions of the embryo, the ectoderm contains numerous Cx43-type junctions. The junctions also link many cells of the mesoderm (m) in this region, including those forming the somites (s). Bars, 50 μm.

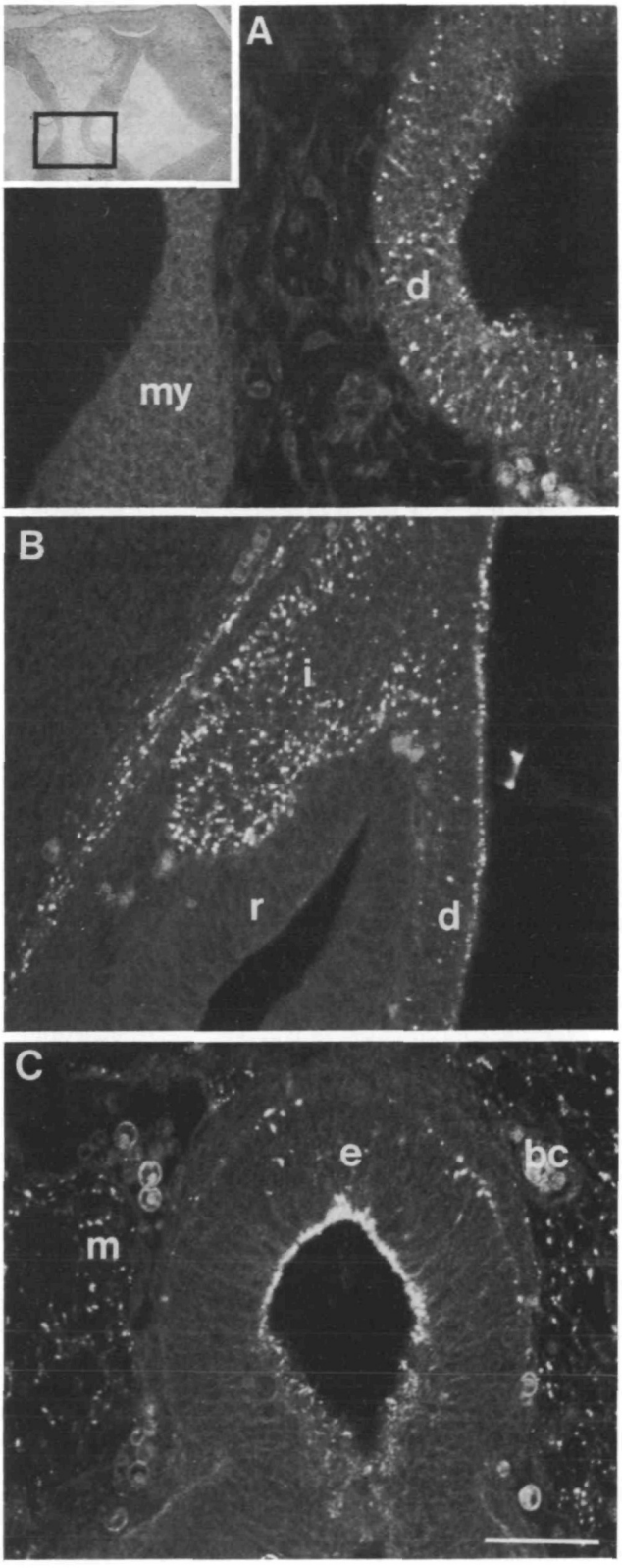


Fig. 3. Expression of Cx43 in brain and its derivatives. The insert shows a low power view of section from an embryo at about 9.5-10 dpc; the boxed area is shown in confocal image in A. A portion of the myelencephalon is visible to the left of the field, the diencephalon, optic vesicle and lens placode (shown at higher magnification in Fig. 4) are at middle and right of the field. (A) From the widespread distribution of Cx43 seen at earlier stages in the head fold and neural plate ectoderm, with establishment of the brain vesicles, the expression of Cx43 becomes restricted to specific areas of the brain. By 9.5-10 dpc, immunoreactive protein is abundant in the diencephalon (d) but undetectable in the myelencephalon (my) as in most other regions of the brain. (B) By 12.5 dpc, Cx43 is highly concentrated in the infundibulum (i) which forms as an outgrowth from the diencephalon (d) in an inductive interaction with Rathke's pouch (r). (C) The epiphysis (e), shown at about 13.5 dpc, also forms as an outgrowth from the diencephalon and also shows a high positive signal as does the surrounding mesenchyme (m). Blood cells (bc) are autofluorescent. Bars, 50 μm.

Immunoreactivity is much higher in the placode than in the surrounding surface ectoderm. The optic vesicle also shows the presence of Cx43-type gap junctions, predominantly in the posterior portion that will become the pigmented layer of the retina. Relatively few junctions are found in the future sensory layer. This pattern of distribution of Cx43 persists at least through 13.5 dpc, except for a rapid and spectacular increase in the signal in the pigmented epithelium (Fig. 4B). Confocal microscopy shows that the very large fluorescent spots represent sites of interaction between the cells of the pigmented epithelium and do not involve contacts with cells of the sensory retina. By day 12-13, staining of the sensory layer of the retina is limited to the anterior portion which is in close proximity to the anterior epithelium of the lens (Fig. 4B). The optic stalk is intensely labeled by the anti-Cx43 antibody. As nerve fibers invade the center of this precursor of the optic nerve (13-13.5 dpc), only the flattened cells at the periphery of the developing nerve are labeled (data not shown).

By 11 dpc, the lens rudiment has invaginated to form the lens vesicle and the cells that form the posterior wall of the vesicle have elongated to form the primary lens fibers. Cx43 is present throughout the vesicle but primarily in the cells that will give rise to the anterior epithelium. Throughout all subsequent stages examined, immunofluorescence is most intense in the anterior epithelium but some small, faint accumulations of immunoreactive protein can be discerned between the fiber cells (Fig. 4C,D). Both corneal epithelium and mesenchyme cells are linked by Cx43 positive junctions (Fig. 4C,D).

Development of the the limb bud

Development of the limbs begins between 9 and 10 dpc. At 9.5-10 dpc, a ridge-like structure has begun to form by thickening of the ectoderm along the ventrolateral margin and tip of the forelimb bud. This is the apical ectodermal ridge (AER) which reaches a peak in development by about 11-11.5 dpc (Fig. 5). There is a remarkable concentration of Cx43-type junctions in the AER from its earliest appearance through its decline. In contrast, at and prior to the peak of development of the AER, Cx43 containing junctions are small and rare

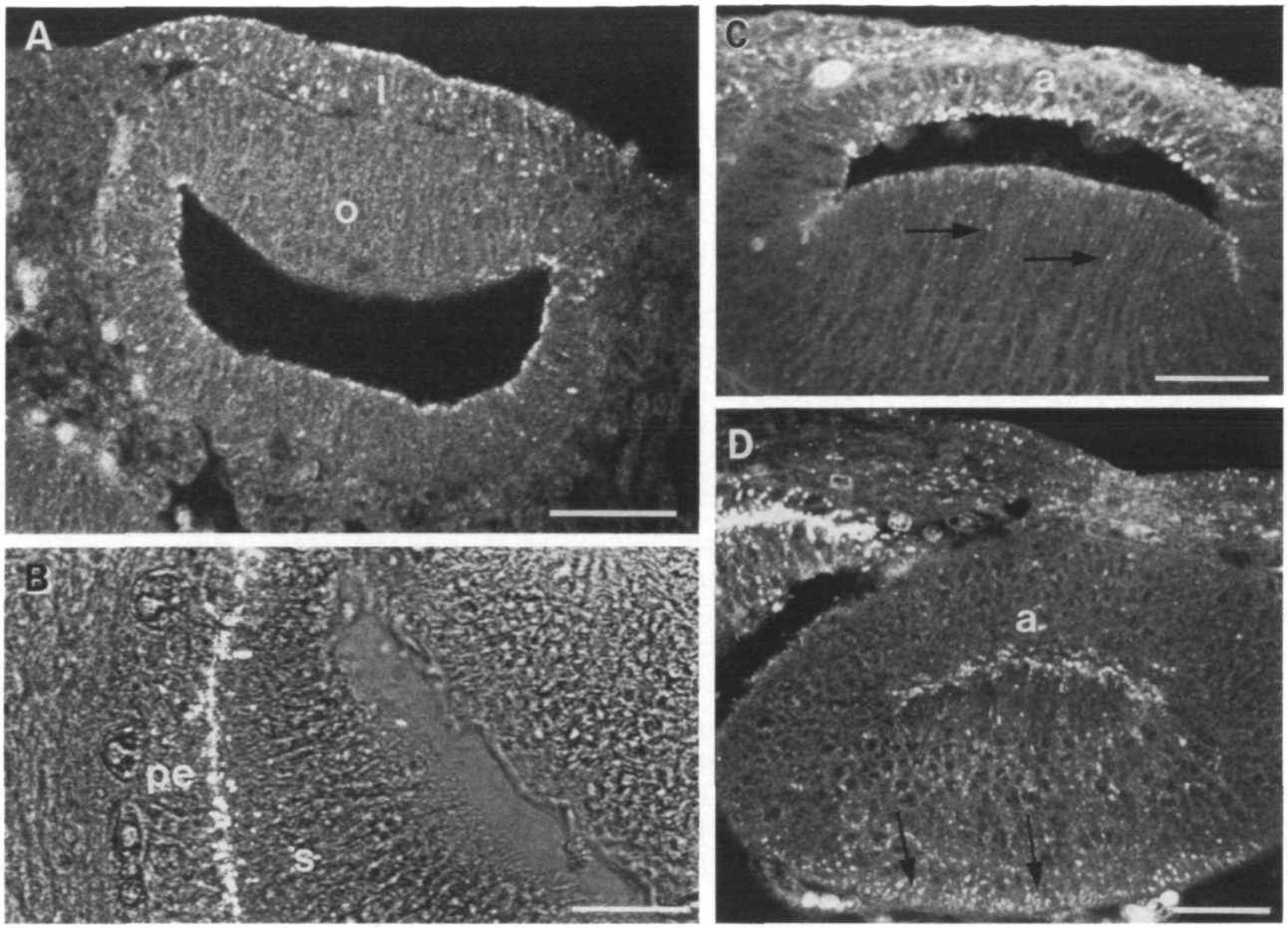


Fig. 4. Expression of Cx43 during morphogenesis of the eye. All the Figs are oriented with anterior to the top. (A) Optic cup (o) and lens placode (l), 9.5-10 dpc. The lens placode (the earliest stage in formation of the lens) shows increased immunoreactivity compared with the surrounding surface ectoderm. The optic cup, which is the progenitor of the retina, also shows the presence of Cx43-type junctions. Bar, 50 μm. (B) Retina, 13.5 dpc. The pigment epithelium (pe) is intensely fluorescent, indicating the presence of large Cx43-type junctions. The sensory layer (s) shows the presence of small junctions only in the region that will become the iris. Bar, 25 μm. (C) Lens, about 12.5 dpc. Cx43 is abundant in the anterior lens epithelium (a) and detectable as small fluorescent spots between the lens fibers (arrows). The developing corneal epithelium and mesenchyme at anterior surface of the lens are also positive. Bar, 50 μm. (D) Lens, 13.5 dpc, oblique section taken near periphery of lens. Expression of Cx43 can still be detected in the lens fibers (arrows) as well as the anterior epithelium (a). Bar, 50 μm.

in nonridge ectoderm of the limb. As the AER regresses this difference is not maintained. By 13.5 dpc, the AER is just recognizable and Cx43-type junctions are abundant throughout the entire ectoderm at the tip of the limb bud. At none of the stages examined is Cx43 detected in the mesodermal core of the limb.

Cardiogenesis

By 8 to 9 dpc, the heart primordia have begun rhythmical contractions (Manasek, 1976; Beddington, 1983); however, Cx43 is not clearly localized to cardiac myocytes until 9.5-10.5 dpc, and then only as rare, small junctions between the cells that form the wall of the developing ventricle (Fig. 6A). As the heart chambers enlarge, a system of trabeculae develops, extending inward from the ventricular wall. It is to cells in this trabecular system that Cx43 is preponderantly localized (Fig. 6B), the signal increasing as cardiogenesis proceeds. No immunolabeling of the cells of the atria is seen until 13.5 dpc, the latest stage examined.

Discussion

We have determined the spatial and temporal patterns of expression of one member of the connexin family of proteins during embryogenesis in the mouse. At least seven different connexins have so far been identified in rodents and there are very likely to be more (Willecke et al., 1991; Hoh et al., 1991). In order to understand patterns of communication in the embryo completely, one would obviously have to know the distribution spatially and temporally of all of these molecules. Even then, the mere presence of gap junctions as demonstrated by immunocytochemistry does not address the

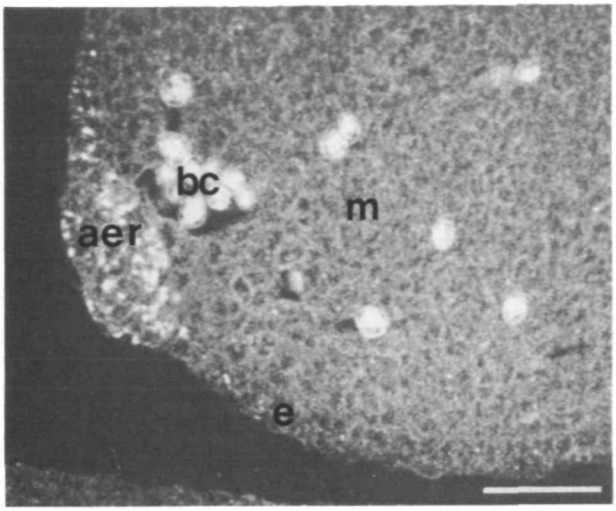


Fig. 5. Distribution of Cx43 in the limb bud, 11-11.5 dpc, the peak of development of the apical ectodermal ridge (aer). Numerous Cx43-type junctions interconnect the cells of the stratified epithelium which forms the ridge. The antibody detects few junctions linking the cells of the nonridge ectoderm (e) and none are found among the cells of the mesodermal core (m). Blood cells (bc) exhibit autofluorescence. Bar, 50 μm.

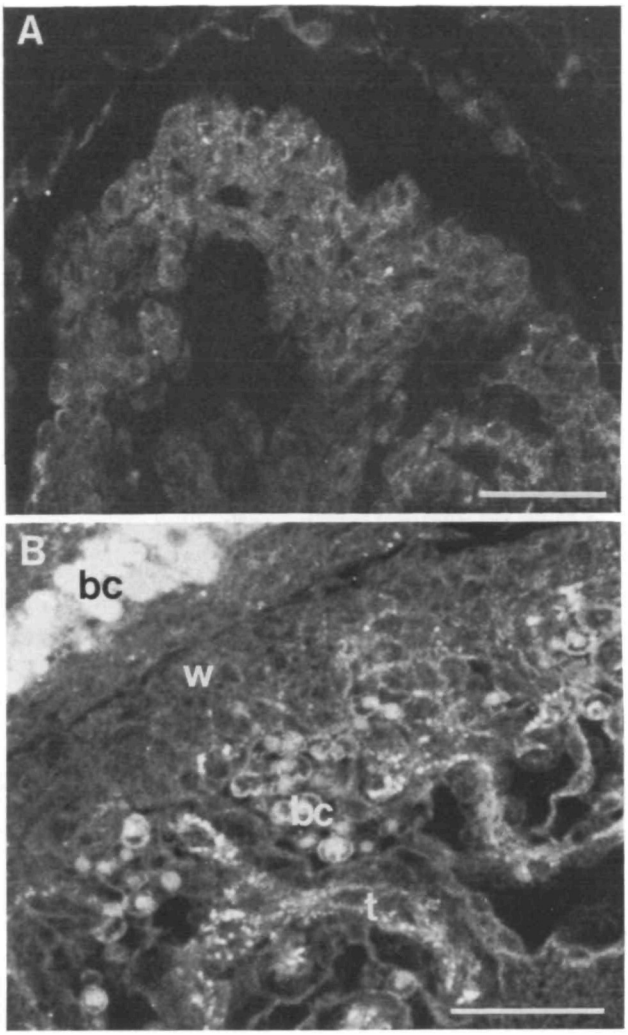


Fig. 6. Expression of Cx43 during cardiogenesis. (A) Ventricle, about 10 dpc. Cx43-type junctions appear few and small. (B) Ventricle, about 12.5 dpc. Junctions have increased markedly in number and are distributed primarily along the trabeculae (t) that extend inward from the ventricular wall (w). The very bright cells are blood cells (bc) which autofluoresce. Bars, 50 μm.

question of their physiological competence. Among other factors that must be considered is the limitation of immunocytochemistry in the detection of very small gap junctions and the possibility that not all immunoreactive material is junctional. In addition, one cannot eliminate the possibility that our antibodies, although highly specific, would interact with an as yet unidentified connexin. As a result, studies such as ours cannot be used to build a model of connectivity in the developing embryo without further data. The patterns observed nevertheless contribute important information, which future studies will amplify. Our results show clearly that throughout development, Cx43 is expressed in complex and changing but highly specific patterns, indicating a tight control of gene expression. It is presumed that the type of connexin determines the physiological behavior of a given gap junction. We may therefore surmise that there are very specific patterns of intercellular exchanges, presumably related to the developmental landmarks through which different regions are passing. Because of the large volume of information we have obtained, we have selected for discussion below only a few areas and stages we believe may be of special interest.

Cx43 is a component of gap junctions in all three germ layers

Our results show that the Cx43 gene is expressed very early after implantation of the mouse embryo. This result confirms and extends the results of others (Barron et al., 1989; Nishi et al., 1991) who have detected Cx43 mRNA and protein in preimplantation mouse embryos.

The few gap junctions identified by the anti-Cx43 antibody at the early egg cylinder stage are not likely to be enough to account for the extensive dye and ionic coupling and numerous gap junctions observed by Lo and Gilula (1979) in their studies of the development of 'postimplantation' mouse blastocysts *in vitro*. Although their *in vitro* conditions and our *in vivo* ones are clearly not equivalent, it may well be that our antibody is not detecting all gap junctions present in the early egg cylinder-stage embryo. The discrepancy may be a matter of detectability; the junctions may be too small to be seen or composed of Cx43 in some configuration not recognized by our antibody. Another possibility is that most of the junctions at this stage are composed of some other member of the connexin family. Lee and colleagues (1987) have used anti-Cx32 antibody to detect protein in mouse embryos by immunocytochemistry at the morula stage and by Western blots of homogenates of morula and blastocysts. However,

Nishi et al. (1991) found no evidence for expression of Cx32 at these stages. Moreover, Barron et al. (1989) detected Cx32 protein but no Cx32 mRNA in isolates from the two-cell stage through late morula. The data are not sufficient to allow us to conclude that any gap junctions in the early egg cylinder are composed of Cx32. In fact, the absence of Cx32 mRNA at earlier stages suggests that this is unlikely.

The relative number of Cx43 expressing cells increases rapidly in the embryo with the separation of the egg cylinder into extraembryonic and embryonic regions and the formation of the mesoderm. At the late egg cylinder or early gastrula stages, we observed nothing in the pattern of localization of Cx43 in the embryonic ectoderm or mesoderm to reflect the communication compartments described by Kalimi and Lo (1988). However, by late gastrula, a stage not examined by Lo and coworkers, subpopulations of mesodermal and endodermal cells can be discerned with the Cx43 antibody as a marker, suggesting that groups of cells have now embarked on divergent pathways. At the end of gastrulation, the ectoderm is the most intensely stained region in the embryo but in this layer there is as yet no evidence of any regionalization in the distribution of Cx43-type gap junctions.

During organogenesis, as developmental pathways are restricted so is the expression of Cx43

In the adult rat, Cx43 is distributed in a wide variety of organs and tissues including the heart, brain, smooth muscle of the vascular wall, eye lens anterior epithelium, retina, cornea, ciliary body, ovarian follicle and corpus luteum, testes, pituitary, intestine, stomach, pineal gland, and kidney (Manjunath et al., 1985; Yancey, unpublished; Dermietzel et al., 1989; Larson et al., 1990; Beyer et al., 1989; Yamamoto et al., 1990). We have found that between 9 and 13.5 dpc when most of the organs are established, the distribution of Cx43 becomes progressively restricted to specific loci, some of which presage the adult distribution, some of which are transient. A feeling for the complexity of these patterns can be gained from Table 1. Our results complement and are generally consistent with those of Nishi et al. (1991) who used S1 nuclease protection assays to examine Cx43 expression at still later stages of development. The overall conclusion from our work is that the gene for Cx43 is expressed in a developmentally regulated fashion in organs and tissues originating from all three germ layers. There is, however, a clear preference for expression in organs of ectodermal and, to a lesser extent, mesodermal origin. The fact that Cx43 gene expression occurs at many sites and is developmentally regulated suggests that gap junctions composed of Cx43 may play multiple roles in embryogenesis.

Cx43 expression is upregulated at sites of inductive interactions

The high level expression we observed in tissues involved in pattern formation through inductive interactions is particularly intriguing with regard to functional implications. These tissues include the AER of the limb bud, the lens and retina, and the infundibulum.

The limb bud has long been used as a model system for the study of pattern formation (Saunders, 1948; Summerbell, 1981). The AER, along with the zone of polarizing activity (Tickle et al., 1975), is necessary for the establishment of the proximo-distal and the anteroposterior axes of the limb. The mesenchyme which underlies the AER is necessary for its induction and the maintenance of its activity. At the same time, the AER is believed to be the source of some factor that maintains the underlying mesenchyme (the progress zone) in the undifferentiated state necessary for normal outgrowth of the limb. The AER also plays some role in digit formation as evidenced by the fact that removal of portions of the AER results in the loss of digits (Rowe and Fallon, 1981). Cx43 is very abundant in the AER throughout its existence as a definitive structure, presumably reflecting the numerous gap junctions described by Kelley and Fallon (1977). Intense labeling of the AER has also been observed by van Kempen et al. (1991). On the other hand, there appear to be few Cx43-type gap junctions in the nonridge ectoderm, and there is no evidence that the gap junctions between the cells of the mesenchyme (Kelley and Fallon, 1978) are composed of Cx43. Therefore, Cx43-type junctions do not appear to play a role in any communication that occurs between the AER and the mesenchyme or in the gap junctional communication between the polarizing region cells and the anterior mesenchyme, a signalling pathway recently dissected in the elegant study of the chick limb bud reported by Allen and coworkers (1990). Communication between the AER and the mesenchyme could, of course, be mediated by junctions composed of some as yet undetected connexin or, in theory at least, by heterologous junctions composed of Cx43 contributed by the AER and some other connexin contributed by the mesenchyme (Swenson et al., 1989). In any case, it is tempting to speculate that the developmentally controlled, high level expression of Cx43 in the AER signals a role for this type of junction in the coordinated activity required for normal morphogenesis of the limb (Kelley and Fallon, 1978). It may also be that the junctional pattern seen is a consequence of the interaction.

A similar argument can be made with regard to Cx43 expression in the infundibulum, the precursor of the neural lobe of the pituitary gland, which forms as an outgrowth of the diencephalon where it contacts Rathke's pouch. Although cells in both of the interacting tissues are ectodermal in origin, only the cells of the infundibulum express Cx43. Yet, as in the limb bud, a reciprocal interaction between the two regions is required for normal development of the the various lobes of the pituitary gland.

The situation is somewhat different with regard to the possible role of Cx43 in the interaction between the optic vesicle and head ectoderm that leads to the induction of the lens (Grainger et al., 1988). Here, both tissues express Cx43 at low levels before the optic vesicle contacts the head ectoderm. Upon induction of

the lens placode, expression of Cx43 is markedly elevated, specifically in the cells of the placode. Although lens formation is a well known example of embryonic induction, the mechanisms involved are not well understood (McAvoy, 1980; Grainger et al., 1988). Direct contact between the interacting tissues does not appear to be necessary for the transfer of the inductive signal (Muthukkaruppan, 1965), suggesting that gap junctional channels are not involved. Nevertheless, the rapid upregulation of the expression of Cx43 in the lens placode could indicate that the junctions may play a critical role in the subsequent morphogenesis of the lens.

Cx43 expression is detected early in development of the heart but is differentially regulated in ventricle and atrium

Cx43-type junctions are found in the vicinity of the heart rudiment at the late gastrula stage but we were unable to determine whether the staining represented the small gap junctions which have been observed between the myocardial plate cells (Navaratnam et al., 1986). We detected Cx43 in the heart ventricle reliably, but at very low levels, by 9.5-10 dpc. Our results are consistent with those of Gros et al. (1978) who found only small and few gap junctions by freeze fracture of 10 dpc heart. They, as we, found evidence for an increase in size and number of the junctions with time. In addition to our results, evidence that the junctions are composed of Cx43 comes from the recent reports of others who have detected Cx43 mRNA in heart ventricle by 11 dpc (Fromaget et al., 1990) and protein by 13 dpc, the earliest stage examined (van Kempen et al., 1991).

We did not detect any Cx43 in the atrium until about $\frac{2}{3}$ of the way through gestation and then the immunofluorescent signal was very low compared with that in the ventricle, i.e., it seems that expression of Cx43 is induced asynchronously in the two major divisions of the developing heart. By birth, we were no longer able to discern any differences in immunostaining that could be related to the different heart chambers (Yancey, unpublished).

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